REMARKS

In the Office Action dated June 17, 2003, the Examiner rejected claims 1, 2, and 10-18. The Examiner withdrew claims 3-9 and 19-26 from consideration.

Following entry of the present amendment, claims 1-15 and 17-26 are pending. In the present amendment, Applicants canceled claim 16 without prejudice or disclaimer and amended claims 1-2, 10-13, and 17-18. The amended claims recite an "archaebacterial" DNA polymerase, which finds support in the specification, for example, at page 10, lines 6-19. No new matter is added by the present amendments. A typographical error in the spelling of the word "homogeneous" was also corrected in claims 10-12.

I. Rejection of Claims 1, 2, and 10-18 Under 35 U.S.C. § 112, First Paragraph

The Examiner rejected claims 1, 2, and 10-18 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. Specifically, the Examiner stated that:

> The specification as filed, is enabled for a DNA polymerase from *Pyrococcus furiosis*, but is not enabled for a DNA polymerase from any and all "samples". The art of biotechnology is a highly unpredictable art and it would be an undue burden for one of ordinary skill in the art to test any and all sources to see if they contained the claimed enzyme.

Office Action at page 3. Applicants respectfully traverse.

A claim is enabled if one skilled in the art can make and use the claimed invention without undue experimentation. Whether experimentation is undue is based, in part, on the guidance provided in the specification and the level of skill in the art. M.P.E.P. § 2164.01 at 2100-178 – 2100-179 (8th ed. rev. 1, 2003). Applicants submit that claims 1, 2, and 10-18 meet the standard of enablement for the following reasons.

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Amended claim 1 recites a method for obtaining "archaebacterial DNA polymerase" from "a sample comprising at least one archaebacterial DNA polymerase." The specification teaches examples of archaebacteria and archaebacterial DNA polymerases. Specification at page 10, lines 9-15. The specification also contemplates "recombinantly-produced archael polymerases that are purified by the novel methods of the invention." Specification at page 10, lines 18-19. The specification also teaches that, "[t]he skilled artisan will appreciate that a variety of starting materials may be employed in the claimed invention. For example, supernatant fluid from cells that include vectors for expressing secreted forms of polymerase may be employed. . . ." Specification at page 15, lines 5-7. The specification also provides detailed examples of the purification of DNA polymerase from the archaebacteria *Pyrococcus furiosis* using chromatography, including Poly U Sepharose chromatography. Specification at pages 16-20. Furthermore, the Examiner fails to explain why one skilled in the art would not recognize that, at the time of filing, a protein of interest could be obtained by purification from a variety of "sources" including, for example, host cells that express natural or recombinant protein. In fact, the enclosed article by Ausubel suggests otherwise. See Ausubel, F.M., et al., Current Protocols in Molecular Biology at pages 10.0.10 – 10.0.14, Figures 10.0.1 – 10.0.4 (Supplement 44, 1998) (a copy of Ausubel is enclosed).

The teachings in the specification and the level of skill in the art at the time of filing provide guidance as to the "sources" from which the claimed archaebacterial DNA polymerase may be obtained. The Examiner provides no evidence that anything more than routine techniques are needed to obtain such archaebacterial polymerase from such sources. Thus, the Examiner fails to establish that one skilled in the art could not

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practice the method of claim 1 without undue experimentation. Claim 1 is therefore fully enabled, as are claims 2 and 10-18, which depend from claim 1. Withdrawal of the rejection of those claims under 35 U.S.C. § 112, first paragraph, is respectfully requested.

II. Rejection of Claims 1, 2, 10-12 and 14 Under 35 U.S.C. § 102(b)

The Examiner rejected claims 1, 2, 10-12, and 14 under 35 U.S.C. § 102(b) as allegedly being anticipated by Bezuglyi, Bernard, or Grandgenett. Office Action at page 4. The Examiner cites these references as allegedly teaching "a DNA polymerase being purified using Poly U Sepharose chromatography." Office Action at page 4.

Amended claim 1 recites a method for obtaining "archaebacterial DNA polymerase" from "a sample comprising at least one archaebacterial DNA polymerase." Bezuglyi discusses purification of DNA polymerase I from the eubacterial mycoplasma, Acholeplasma laidlawii. Bernard discusses purification of DNA polymerase from the T-47D human breast cancer cell line. Grandgenett discusses purification of a viral DNA polymerase subunit. None of the cited references teach the purification of an "archaebacterial" DNA polymerase as recited in claim 1. Thus, the cited references fail to disclose each and every limitation of claim 1 and claims 2, 10-12, and 14, which depend from claim 1.

Because the cited references fail to disclose each and every limitation of claims 1, 2, 10-12, and 14, the cited references fail to anticipate the claims. Thus, Applicants respectfully request withdrawal of the rejection of those claims under 35 U.S.C. § 102(b).

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III. Rej ction of Claims 1, 2, and 10-18 Under 35 U.S.C. § 103(a)

The Examiner rejected claims 1, 2, and 10-18 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Bezuglyi, Bernard, or Grandgenett. Office Action at page 4. The Examiner stated that, "[i]t would have been obvious to use the specific types of polymerases and cells since such cells and enzymes are well known in the art. To use such enzymes and cells are simply the choice of the artisan in an effort to optimize the desired results." Office Action at page 4.

To establish a prima facie case of obviousness, the cited references must teach or suggest all the claim limitations. M.P.E.P. § 2142-43 at 2100-123 – 2100-125. As discussed above, none of the cited references, either singly or in combination, teach or suggest all the claim limitations, particularly an "archaebacterial" DNA polymerase.

Furthermore, the examiner must provide some motivation or suggestion to modify or combine the cited references. *Id.* The Examiner has failed to meet this burden. In fact, it is the Applicants' own disclosure that provides such a suggestion or motivation. See, e.g., specification at page 4, lines 6-14, disclosing a motivation to obtain highly purified archaebacterial polymerase for the purpose of optimizing PCR. The Examiner may not rely on the Applicant's own disclosure to supply the necessary motivation. M.P.E.P. § 2143 at 2100-125.

Thus, the Examiner has failed to establish a prima facie case of obviousness. Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1, 2, and 10-18 under 35 U.S.C. § 103(a).

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CONCLUSION

Applicants respectfully request the reconsideration and reexamination of this application and the timely allowance of the pending claims. In the event that the Examiner does not find the claims allowable, Applicants request that the Examiner contact the undersigned at (650) 849-6620 to set up an interview.

Please grant any extensions of time required to enter this response and charge any additional required fees to Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: October 16, 2003

By: Danielle Pasqualone REG NO 43847 for
M. Paul Barker
Reg. No. 32,013

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FULL CONTENTS OF SUPPLEMENT 44

Instructions for Adding Pages to Core Manual

Full contents for Supplement 44 are listed below, with instructions for removing old pages and inserting new pages. Every page included with Supplement 44 is labeled as such beneath the page number.

Remove pages	Insert pages	Subject	Primary change/notes
VOLUME 1			
vii-xii	vii-xii	Main table of contents	Reflects new and revised *UNITS 10.9, 10.10, 10.18, 14.10, & 14.11*
6.11.1-6.11.2	6.11.1-6.11.2	MAb for expression cloning	Revision
VOLUME 2			
CH 10: 3-5	CH 10: 3-5	Chapter 10 contents	Reflects new and revised *UNITS 10.9, 10.10, & 10.18*
10.0.1-10.0.20	10.0.1-10.0.20	Chapter 10 introduction	Revision
10.8.13-10.8.14	10.8.13-10.8.14	Immunoblotting & immunodetection	Revision
10.9.1-10.10.6 (17 pages)	10.9.1-10.10.30 (62 pages)	Gel-filtration chromatography *and* Ion-exchange chromatography	New *UNITS 10.9 & 10.10*
10.13.6-10.13.7	10.13.6-10.13.7	Ion-exchange HPLC	Revision
10.18.1-10.18.9	10.18.1-10.18.10	Metabolic labeling of amino acids	Revised *UNIT 10.18*
14.0.1-14.0.2	CH 14: 1-2[a]	Chapter 14 contents	Reflects new *UNITS 14.10 & 14.11*
14.0.3-14.0.4	14.0.1-14.0.2	Chapter 14 introduction	Revision
—	14.10.1-14.10.11	Principle and application of fluorescence microscopy	New *UNIT 14.10*
—	14.11.1-14.11.12	Basic confocal microscopy	New *UNIT 14.11*
VOLUME 3			
—	**Color Plates tab**[b]		**Insert before Appendices tab**
—	**2 color pages**[b]		**Insert behind Color Plates tab *or* with corresponding units**
A.1B.1-A.1C.1 (2 pages)	A.1B.1-A.1C.1 (2 pages)	Useful measurements and data	Revision
Supps. 42-43 index (3 pages)	Supps. 42-44 index (5 pages)	New supplement index	New entries covering Supplements 42, 43, & 44

[a] Tables of contents and introductions are being gradually converted to a new numbering system as new material is added.

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rally occurring protein, (7) containing a natural leader peptide that would normally be processed, (8) as a fusion protein with a peptide that is not natural to the protein, or (9) lacking glycosylation or other posttranslational modification, or modified incorrectly. Possibilities (1) to (5) affect the method of extraction used to obtain the starting material for purification. Cases (6) to (9) can affect the methods used for purification.

The scheme for purifying soluble recombinant proteins is outlined in Figure 10.0.1. The first stage is to obtain a clarified solution containing the desired protein, with as little in the way of unwanted proteins as possible. For soluble cytoplasmic proteins, case (1), it is not normally possible to exclude any significant amount of unwanted soluble proteins, but in cases (2) to (5) th compartmentalization away from the cytoplasm allows such separation in the initial stage.

It may be necessary to carry out a concentration step before proceeding, especially if the protein has been excreted into the culture me-

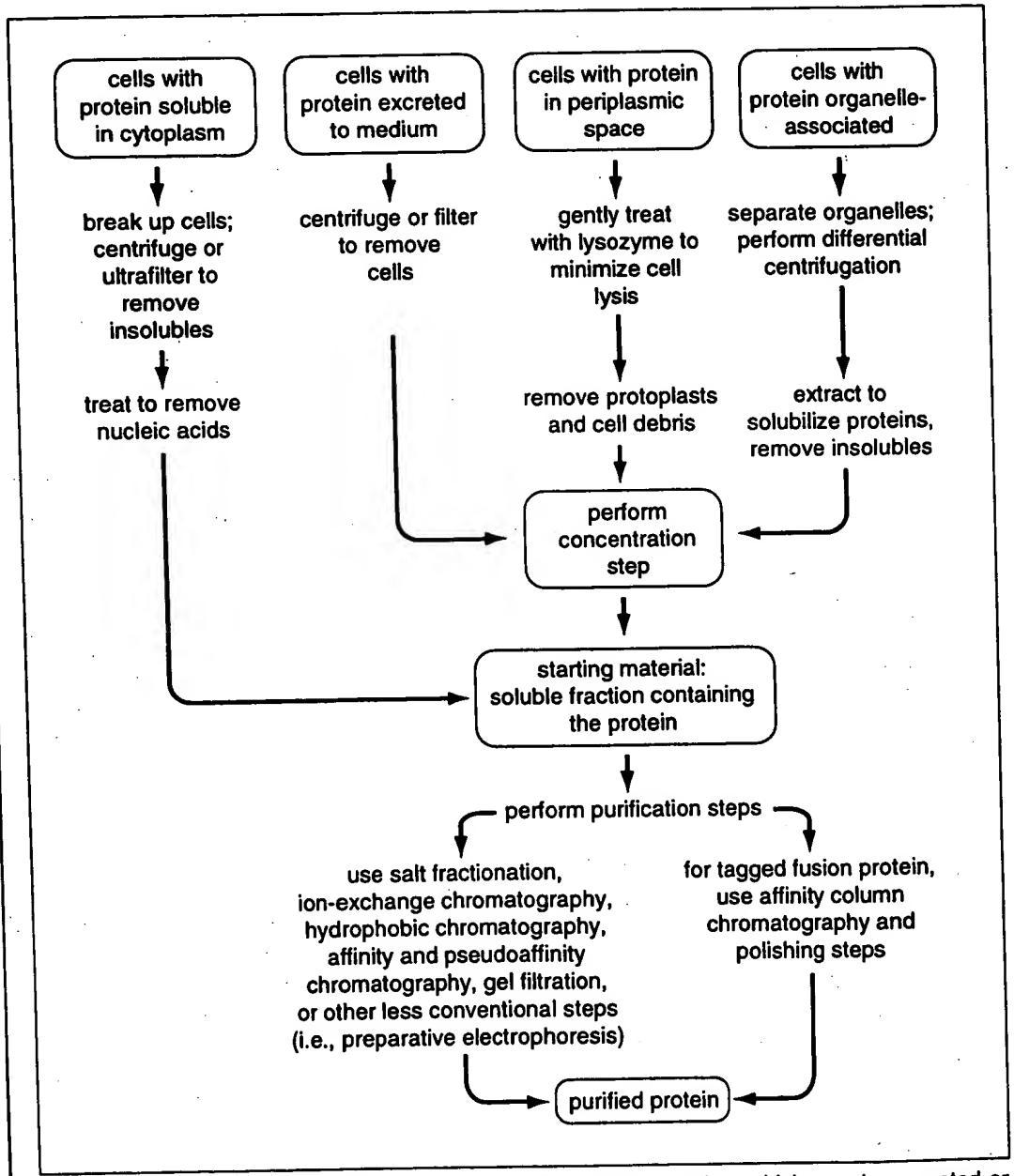

Figure 10.0.1 Purification scheme for soluble recombinant proteins, which may be xcreted or located in the periplasm, in the membrane fraction, or most commonly the cytoplasm. The first step is to obtain an extract containing the desired protein in soluble form. After this, conventional purification steps may be carried out, or affinity purification of tagged fused proteins can be performed.

dium. Normally ultrafiltration is used (see *APPENDIX 3C*), although other techniques are possible, especially if the extract contains particulates that block ultrafiltration membranes.

Recombinant expression in the cytoplasm of bacteria, followed by extraction via total cell disruption, results in large amounts of nucleic acids being solubilized with the protein. A number of treatments to remove nucleic acids are possible. Streptomycin is used to precipitate ribosomal material, and cationic polymers such as protamine (a basic protein) and polyethylenimine will form insoluble complexes (at low ionic strength) with nucleic acids. In addition, viscosity caused by DNA can be reduced by adding small amounts of DNase.

Insoluble Recombinant Proteins

It has been found that many proteins do not fold correctly when expressed in bacteria (mainly in *E. coli*), and as a result aggregation occurs, leading to large insoluble inclusion bodies within the cytoplasm of the cells (see Chapter 6 of *CPPS*). Although this creates major difficulties in obtaining satisfactory amounts of active native product, it greatly simplifies the initial stage of purification.

The purification scheme for recombinant insoluble proteins is outlined in Figure 10.0.2. After cell disruption, inclusion bodies can be obtained in a fairly pure state by differential centrifugation. They must then be solubilized, however, and the active protein generated by encouraging correct folding. Solubilization is

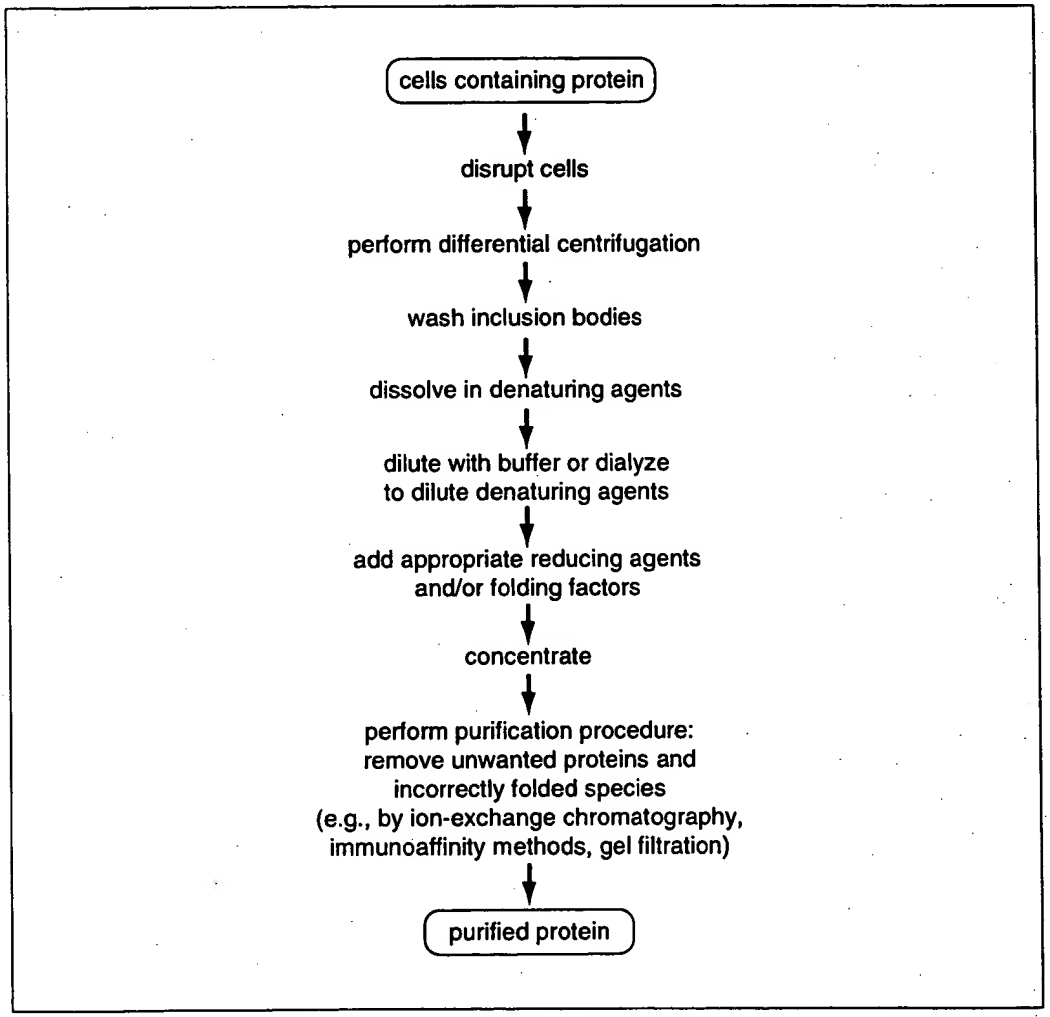

Figure 10.0.2 Purification scheme for insoluble recombinant proteins that are produced as inclusion bodies in the cytoplasm of host cells. The cells must be broken open, and then th insolubl inclusion bodies separated by differential centrifugation. Solubilization is achieved by the use of denaturing solvents, and renaturation of the dissolved protein occurs on removal of the denaturant. Further polishing steps will be needed to remove small amounts of contaminating proteins as well as incorrectly folded species. Additional information can be found in *Current Protocols in Protein Science* (see Palmer and Wingfield, 1995; Pain, 1995; Wingfield et al., 1995).

usually accomplished with guanidine hydrochloride and/or urea, and thiols such as 2-mercaptoethanol or glutathione are included to disrupt any disulfides that have formed and prevent more from forming. Folding the protein correctly may require a variety of additions to the solution, as well as slow removal of the denaturant. The latter can be carried out by simple dilution or by dialysis (*APPENDIX 3C*). Folding occurs best at low protein concentrations, so dilution may be adequate. If the native protein does contain disulfides, then it is important to create redox conditions such that some (but not excessive) oxidation of thiols can occur. A combination of oxidized and reduced glutathione is commonly used. In addition, the action of the enzyme protein disulfide isomerase, which can make and unmake disulfides by exchange reactions, has been found to be beneficial in many cases. If the native protein is of intracellular origin, it probably will not contain disulfides; it will, however, contain cysteines, so a full reducing potential should be maintained. Specific methodology is discussed in *CPPS* (Wingfield et al., 1995; Wingfield, 1995a).

Not all proteins can fold unassisted by other cellular components. Chaperonins are proteins whose role is to assist in folding proteins including those unfolded by heat shock (Zeilstra-Ryalls et al., 1991). The ones most studied, which are just becoming commercially available as of 1996, are the *E. coli* chaperonins GroEL and GroES, both of which are needed, together with ATP, to renature many proteins. Proline residues can adopt two isomeric conformations in proteins, and the wrong conformation is switched to the correct one by the enzyme prolyl isomerase, aiding the process of protein folding. At present these are not large-scale prospects, both because of the cost of the chaperonins and because the agents operate best in vitro at very low protein concentrations.

Once the proteins are folded, the purification process consists of removing small amounts of still incorrectly folded protein plus any other host proteins that were trapped with the original inclusion bodies. The former may be difficult, as incorrectly folded species have a size and charge similar to those of the correct product. However, subtle differences arising from the folded conformation can be exploited by chromatographic techniques. In ideal cases immunoaffinity techniques using antibodies specific for either the incorrectly folded form or the correct one can be used to resolve the mixture.

Soluble Nonrecombinant Proteins

There are so many sources of soluble proteins that it is not possible to give a complete overview of methods used to obtain starting extracts from which a desired protein can be isolated. The sources can be classified as either microorganisms, plants, or animals, as shown in Figure 10.0.3, but these in turn should be subdivided according to how the starting extract is obtained. In particular there is a distinction between extracellular and intracellular proteins. With the latter it is necessary to disrupt the cells and release the proteins, whereas with the former, if the extracellular fluid can be obtained directly, there need be no contamination with intracellular proteins. Extracellular sources include microorganism culture medium, plant and animal tissue culture medium, venoms, milk, blood, and cerebrospinal fluids. Soluble proteins may also occur within organelles such as mitochondria; these may best be obtained by first isolating the organelle, then disrupting it to release the contents.

The starting extract normally contains between 5 and 20 mg protein per milliliter, though lesser concentrations can be dealt with, especially if working on a small scale. It may be necessary to include a concentration step before starting the purification process in order to approach that level. There are exceptions to every rule, however, and very high protein concentrations can be handled, for example, with two-phase partitioning (Walter and Johansson, 1994). When isolating proteins on a large scale, the volumes being manipulated become of increasing concern, so maximizing protein concentration can be an important aim. The starting extract should be clarified, usually by centrifugation; on a large scale, ultrafiltration methods (*APPENDIX 3C*) are becoming more widely used. Pretreatment of certain extracts to remove excessive amounts of nucleic acids, phenolics, and lipids may be necessary in order to obtain an extract that is amenable to standard fractionation procedures.

Fractionation procedures can somewhat arbitrarily be divided into three steps: initial fractionation, secondary fractionation, and polishing. In initial processing, which deals with a large amount of extract that is not all protein, materials may become soiled and may be unable to be used many times. Consequently, methods that do not require expensive reagents or adsorbents are preferred. Classic salt fractionation and the less-used organic solvent fractionation can achieve, if not a high degree of

purification, a useful level of concentration and removal of much unwanted nonproteinaceous material. Alternatively, a highly selective affinity procedure may be used as the first step, but only if the affinity material is inexpensive to make and/or the extract is a simple, clear solution as opposed to a turbid whole-cell homogenate.

Secondary processing achieves the main purification, and in difficult situations may involve two or more steps. Ion-exchange (*UNIT 10.10*) and hydrophobic-interaction chromatography, gel filtration (*UNIT 10.9*), and affinity techniques (*UNITS 10.11B & UNIT 20.2*) are the main procedures. Finally, it may be necessary to remove traces of contaminants by "polishing"

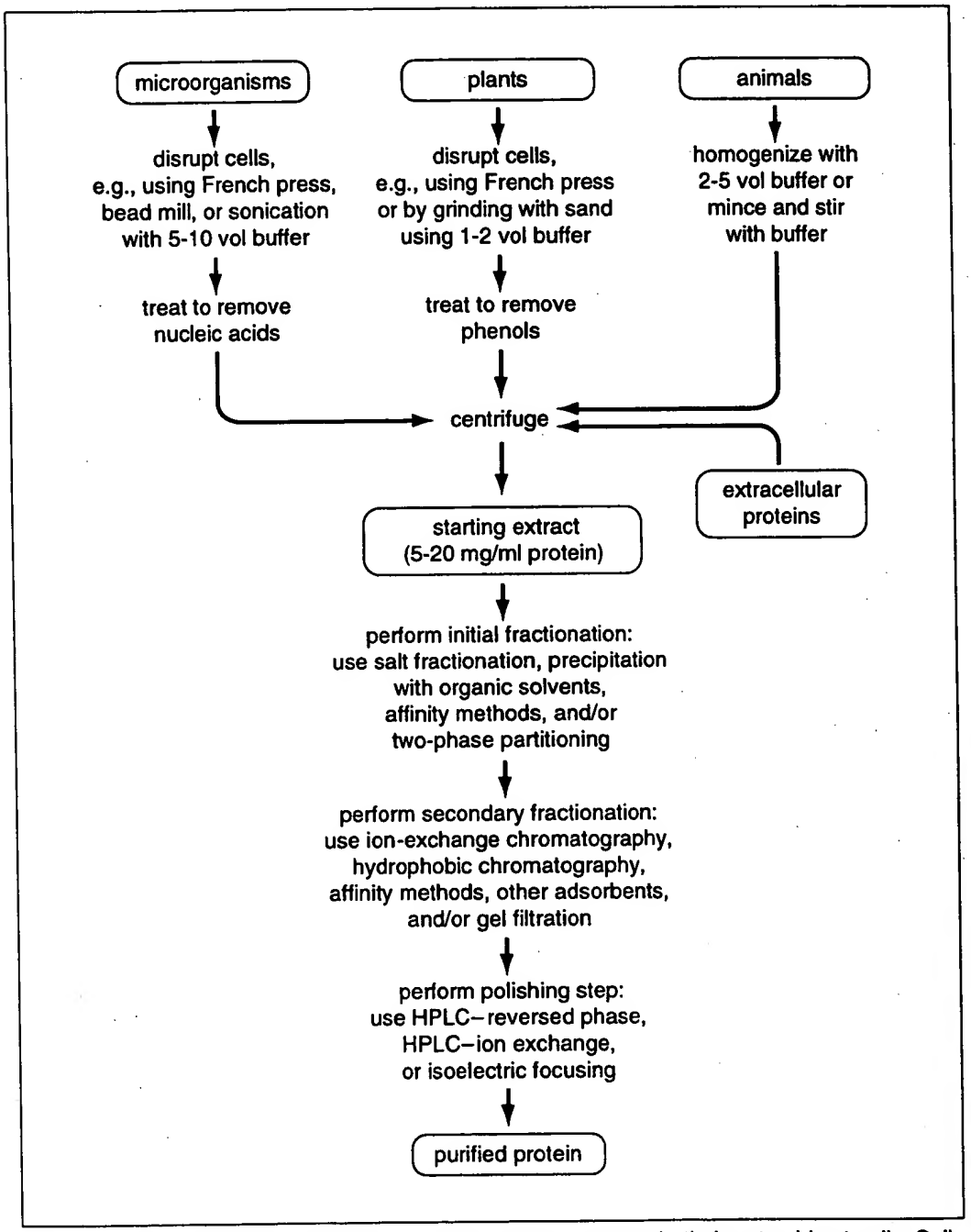

Figure 10.0.3 Purification scheme for soluble proteins present in their natural host cells. C lls must be disrupted to release the proteins, usually in the presence of 2 to 10 ml of a suitable buffer per gram weight. After removal of insoluble material, the process will generally require several steps, using various standard fractionation procedures in a suitable order. For production of highly pure protein, a final polishing step may be required to remove final trace contaminants. Additional information can be found in *Current Protocols in Protein Science* (see Wingfield, 1995b).

using high-resolution procedures such as RP-HPLC (*UNIT 10.12*) and isoelectric focusing (IEF; *UNITS 10.3 & 10.4*). Because every protein has unique characteristics, it is impossible to make general statements about procedures to be followed.

Membrane-Associated and Insoluble Nonrecombinant Proteins

Proteins that are not physiologically soluble can be purified after extracting and removing soluble proteins, thereby achieving a substantial degree of purification at the extraction step (Fig. 10.0.4; also see Wingfield, 1995b,c, in *CPPS*). To carry out a purification it is nearly always necessary to obtain the desired protein in a soluble form, which will often require the addition of solubilizing agents such as detergents. Some proteins remain insoluble even with detergent treatment, and so can be substantially purified by removing the soluble fractions. Some membrane-associated proteins be-

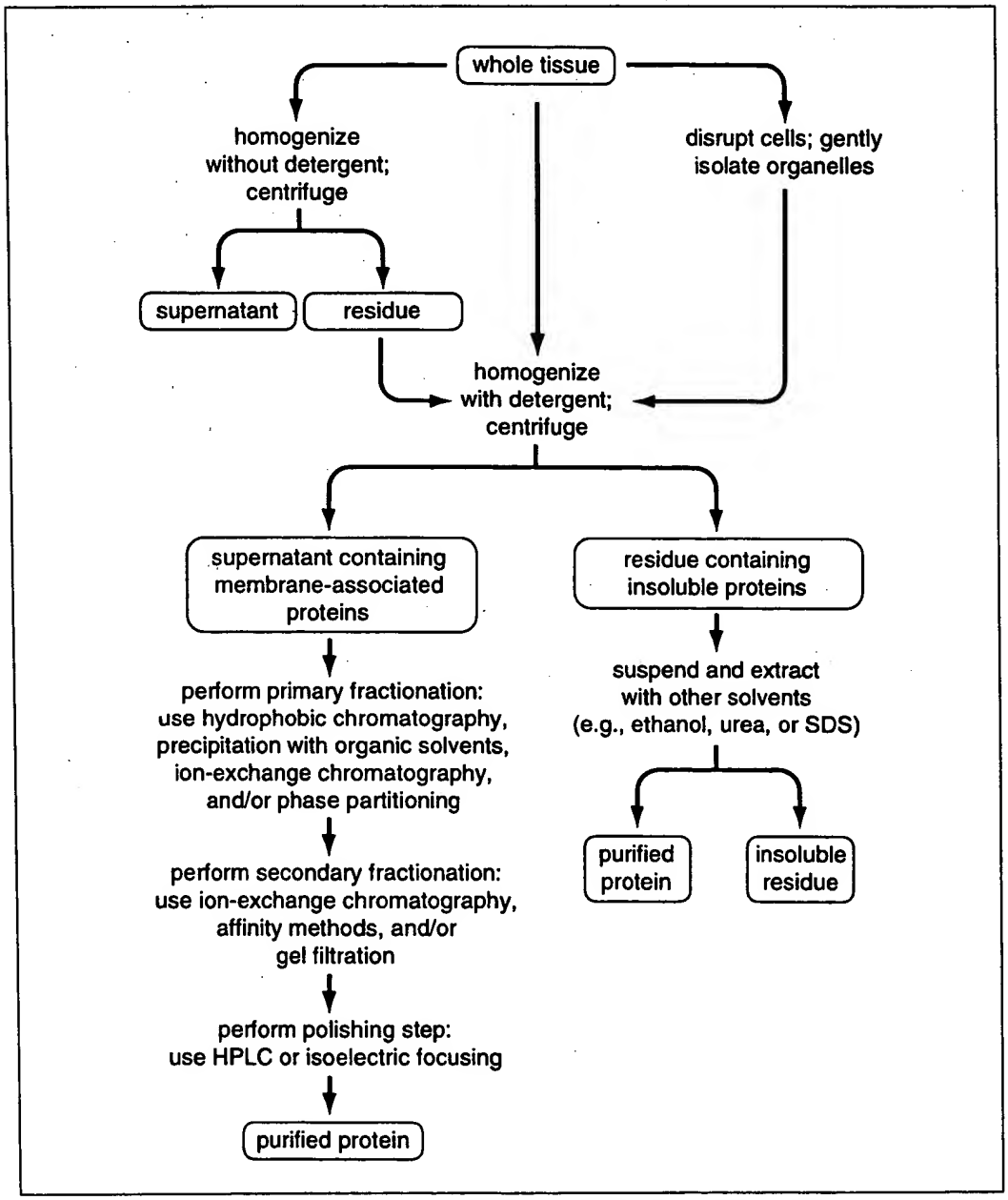

Figure 10.0.4 Purification scheme for membrane-associated and poorly soluble proteins (nonr - combinant). An initial purification can be achieved by isolation of organelles containing the desired protein. Membrane proteins are normally solubilized with a nonionic detergent, although loosely associated proteins may be extracted without detergent at high pH, with EDTA, or with small amounts of an organic solvent such as *n*-butanol. Normal fractionation procedures may need some modification if the detergent is required throughout to maintain the integrity of the protein.